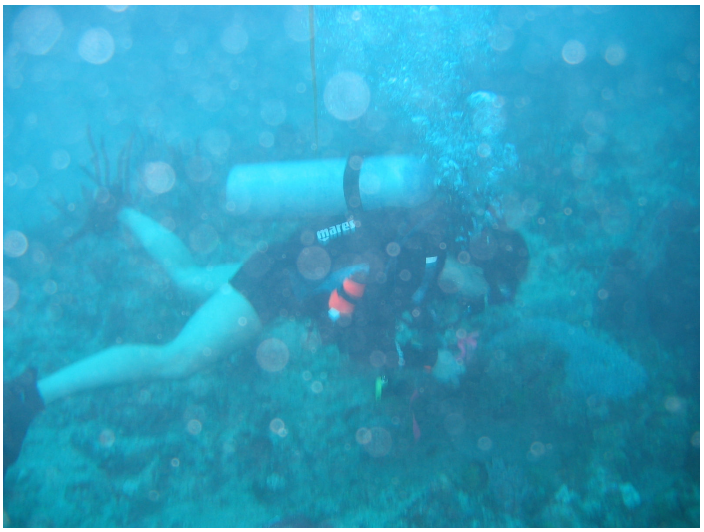
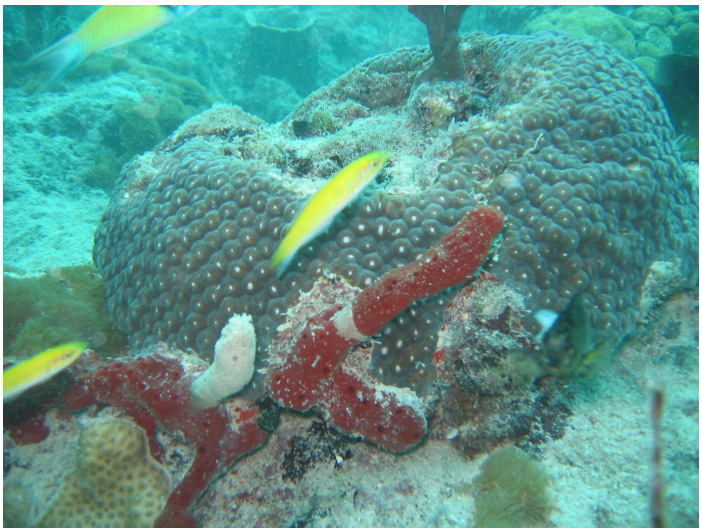
Miami ODMDS
Coral Stress Gene Expression Study

Final Report
Miami, FL – Dade County





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Miami ODMDS Coral Stress Gene Expression Study

This report summarizes the collections made for the Miami ODMDS Coral Stress Gene Expression Study. Within the past few years, the US Army Corps of Engineers and Port of Miami have commenced a project to widen and deepen the Miami harbor and channel. Material from the dredging is processed and dumped offshore. During disposal, a plume of sediment disperses and settles to the bottom where it covers sessile organisms. Sediments (suspended or deposited) are almost universally recognized as having inhibitory effects on coral reef communities. The purpose of this project is to investigate whether the effects of ocean disposed dredge material has any detectable effect on corals using genetic biomarkers of stress.

Sample Collection

The offshore disposal of dredged material off the coast of Dade County began on July 11, 2005. A reconnaissance survey and initial collection of the coral was made in August 2004. Colonies were mapped and photographed at each site and sites were marked with underwater buoys and GPS coordinates. During the next collection trip, February 22nd – 24th, 2005, permanent sites were established including South 1 (60 ft depth), South 2 (30 ft), North 1 (30 ft), and Reference 1 (30 ft) (Table 1). A subsurface buoy was placed at each site, GPS coordinates were recorded and 4 or 5 colonies of *Montastrea cavernosa* were marked with a numbered tag and photographed. The samples collected on this trip should be considered baseline because disposal of dredged material had not been initiated at the time of sampling. Sampling sites were selected based on models of the sediment disposal plume and the areas of anticipated contact with living corals (Figs. 1 & 2). In June 2005 (20th – 22nd) colonies from previously marked sites were photographed and resampled and Reference Site 2 (60') was established, photographed, and sampled (Table 1). These collections, in addition to the previous collections, represent baseline conditions.

The fourth sampling occurred in August 2005 (16th – 18th) after dumping commenced in July. All colonies were photographed and resampled. The samples collected on this trip represent potentially impacted coral from dumping (Fig. 3). A second impact sampling occurred during October 10th – 13th, 2005. All colonies were photographed and resampled. The last sampling was done during June 19th – 22nd, 2006. This sampling represents a third impact collection and occurred after an extended period of discontinued dumping, but immediately after dumping resumed (Fig. 4). Refer to Table 1 for site summary and Figure 5 for pictures of representative colonies from each site.

Temperature and Dumping Data

The amount of dredge material dumped each day was measured as the difference in draft feet, the vertical distance from the bottom of the hull to the waterline, between a full barge at departure and an empty barge after dumping. Dumping of dredge material began on July 11th, 2005 and commenced for four and a half months, temporarily stopping on November 11th, 2005. No dumping occurred from November 11th, 2005 until March 24th, 2006 at which point dumping started again for a brief period through April 13th, 2006. The final phase of dumping began on June 5th, 2006 and ended on July 20th, 2006 (Fig. 4). Ocean temperature data was recorded using

the EPA's Acoustic Doppler Current Profiler (ADCP). Measurements were taken near South Site 1 (20 m) a few feet off the bottom (Fig. 4).

Materials and Methods

Colonies were sampled for gene expression study from all sites and all dates (~1 cm^2 preserved in TRIzol). Histopathological examination was also performed by Dr. Bernardo Angel-Vargas on samples collected in February from South Sites 1, South Site 2 and from the North Site (~4cm^2 preserved in Z-fix).

Coral samples preserved in TRIzol® were processed for RNA extraction following the manufacturer's protocol (based on Chomezynski and Sacchi, 1987). RNA was purified using a silica-gel–membrane column, checked for quality by formaldehyde gel electrophoresis and quantified with a UV spectrophotometer. At least 5 ug of the purified RNA was reverse transcribed and labeled using amino-allyl incorporation and enzymatic annealing of a fluorescent dye. Labeled cDNA from each coral sample was hybridized to a minimum of two microarrays following the manufacturer's protocol (CombiMatrix). A Perkin-Elmer scanner was used to detect the fluorescence of each spot and software provided by CombiMatrix was used to analyze signal intensity for the oligonucleotides. Signal intensities were normalized to control spots on the array and analyzed using JMP statistical software (SAS Institute, Inc). Levene's test checked for equality of variance of each gene between sites and between dates. Since the experimental design is unbalanced, analysis of variance (ANOVA) in the form of a general linear model (GLM) was used to determine significant differences between samples when variances were equal. If the variances were unequal, the Kruskal-Wallis test was used to determine significant differences. Tukey's HSD post-hoc test was then performed to determine which date or site was significant within a group. The stress focused microarray used to analyze patterns of coral gene expression consisted of oligonucleotides that represent 150 Anthozoan genes involved in oxidative stress, cellular redox reactions, protein folding, membrane repair, cell maintenance and a variety of other processes involving stress response and homeostasis. A more detailed description of materials and methods can be found in Edge, S., *in prep*.

Results

The results of the histopathology study reveal that all corals show some level of stress. South Site 1 and the North Site exhibit higher degrees of tissue damage than South Site 2 (Appendix A).

Reference Site 1 shows an overall lower level of expression across all genes than any other site and expression is lower in February 2005 than any other date (Figs. 6 & 7). Gene expression analysis of the 153 genes represented on the array revealed 88 genes differentially expressed between dates or sites and 65 genes that showed no significant difference in expression across samples. Genes were clustered into two broad categories based on cellular function using Gene Ontology (GO; www.geneontology.org). Genes involved in homeostasis, metabolism, respiration and energy production were grouped into the "non-stress" category the rest of the genes were place in the "stress" category. Stress gene were further clustered by Gene Ontology into nine subgroups including general cellular defense response, heat shock functions, response to high light, response to inflammation and wounding, response to low light or shading, oxidative stress,

regulation of apoptosis, response to ultraviolet radiation and response to xenobiotics or heavy metals (Table 2).

Relative gene expression determined by statistical analysis was compared between dates and sites for stress and non-stress genes that showed elevated, decreased or non-significant expression (Fig 8 & 9). Patterns of relative gene expression are similar between February 2005 and June 2005 across all sites, while August 2005 and October 2005 share a similarity in expression pattern (Fig. 8a & 8b). June 2006 is unique in exhibiting the largest percentage of differentially expressed genes at all sites compared to other dates (Fig 8c). Sites 2 through 5 exhibit similar relative expression across dates, while Site 1 stands out as having the largest percentage of differentially expressed genes (Fig. 9a & b). This is further evident by the percent of relatively elevated stress genes by site (Fig. 9c).

An observation of the relatively elevated stress genes by subgroup reveals different patterns of expression between sites and dates. During the first four collection months, South Site 1 has more elevated stress genes than other sites (Figs. 10a – 13a). June 2006 is an exception showing uniform activity of elevated stress genes by subgroup across all sites (Fig. 14). February 2005 and June 2005 share a similar pattern of elevated stress gene expression, while August 2005 and October 2005 are comparable. The difference in number of elevated stress genes between the south sites and reference sites is less extreme in February and June compared to August and October (Figs. 10 – 13). The bulk of elevated stress genes in August and October are represented at South Site 1 (Figs. 12a & 13a). In October 2005 no stress genes are elevated at Reference Site 2 (Fig. 13).

Discussion

The results of this study show that different gene expression patterns are observed between sites and collection dates. Results from the two baseline collection dates prior to dumping are similar (Feb and June 05); likewise gene expression levels from the first two dates post-dumping are similar (Aug and Oct 05). This is consistent with the hypothesis that corals in this study are responding to sediment stress. In addition, elevated gene expression patterns from August and October show a site specific response. South Site 1 is the site where the greatest sediment impact is expected because it is in the sediment dispersal plume. This site has more stress genes elevated in August and October than any other site. The classes of elevated stress genes are involved with cellular defense, chaperon or protein folding activity, oxidative stress, xenobiotics or heavy metal exposure, and response to low light environments.

While August and October show site-specific patterns of stress gene expression, the expression patterns from June 2006 are indicative of global stressors. Elevated stress gene expression is uniform across all sites indicating that corals from each location are experiencing stress. Because stress is observed at all sites including the reference sites, the stressor is most likely a global environmental factor such as fluctuations in temperature or salinity. Alternately, several stressors, including sedimentation, may be synergistically impacting the corals. Although sediment may be affecting the corals at impacted sites in June 2006, it is difficult to distinguish a specific response the apparent global stress. It is interesting to note that more genes involved in light harvesting, response to shading, photosynthesis and inflammation and wounding are

elevated at South Site 1 than any other site during June 06. This is consistent with the hypothesis that the impact of the global stressor may be greater when sediment stress is added.

Conclusions

The results of this study are consistent with the hypothesis that dredged sediments come into contact with corals at Sites 1 and 2 and that corals respond with elevated expression of several stress genes. In addition, there are times of the year when corals at all sites seem to be stressed by a global stressor like temperature. Adding sediment stress during periods of global stress appears to elevate the expression of specific stress genes associated with oxidative stress and sensitivity to high light intensity. This suggests that disposal of dredged sediments should be scheduled during periods of low environmental stress, usually winter and spring months for Miami reefs. Corals already experiencing a global stressor like high temperature may not be able to handle the additional effects of sediment stress. The data from this study are undergoing further analysis and will be published in a refereed scientific journal and incorporated into the Georgia Tech Ph.D. dissertation of Sara Edge. Please refer to Edge et. al, *in prep* for a more detailed description of this study.

Table 1. Miami ODMDS Coral Gene Expression Study collection site summary.

GPS	Site	Established	Depth	Species	Colony #'s	Notes
25 42.930'N	South Site 1	February '05	20m	*M. cavernosa*	101	
80 05.385'W			20m	*M. cavernosa*	102	
			20m	*M. cavernosa*	103	
			20m	*M. cavernosa*	104	
25 43.517'N	South Site 2	February '05	10m	*M. cavernosa*	105	
80 05.993'W			10m	*M. cavernosa*	106	missing 6/21/05
			10m	*M. cavernosa*	107	
			10m	*M. cavernosa*	108	
			10m	*M. cavernosa*	109	missing 6/20/06
			10m	*M. cavernosa*	126	added 6/21/05
25 48.696'N	North Site 1	February '05	10m	*M. cavernosa*	110	
80 05.936'W			10m	*M. cavernosa*	111	
			10m	*M. cavernosa*	112	
			10m	*M. cavernosa*	113	missing 6/20/06
			10m	*M. cavernosa*	114	
25 57.569'N	Reference 1	February '05	10m	*M. cavernosa*	115	dead 6/21/05
80 06.028'W			10m	*M. cavernosa*	116	
			10m	*M. cavernosa*	117	bleach 8/16/05 missing 6/20/06
			10m	*M. cavernosa*	118	
			10m	*M. cavernosa*	119	
			10m	*M. cavernosa*	125	added 6/21/05
25 56.701'N	Reference 2	June '05	20m	*M. cavernosa*	120	missing 6/20/06
80 05.334'W			20m	*M. cavernosa*	121	
			20m	*M. cavernosa*	122	
			20m	*M. cavernosa*	123	
			20m	*M. cavernosa*	124	

Figure 1. Disposal plume and study site map. Sites were chosen based on disposal plume estimates. Red circles indicate the sites. Depth is recorded to the right of the image for each site. Miami ODMDS Disposal Plume Advection Estimates for a travel time of 5 hours.

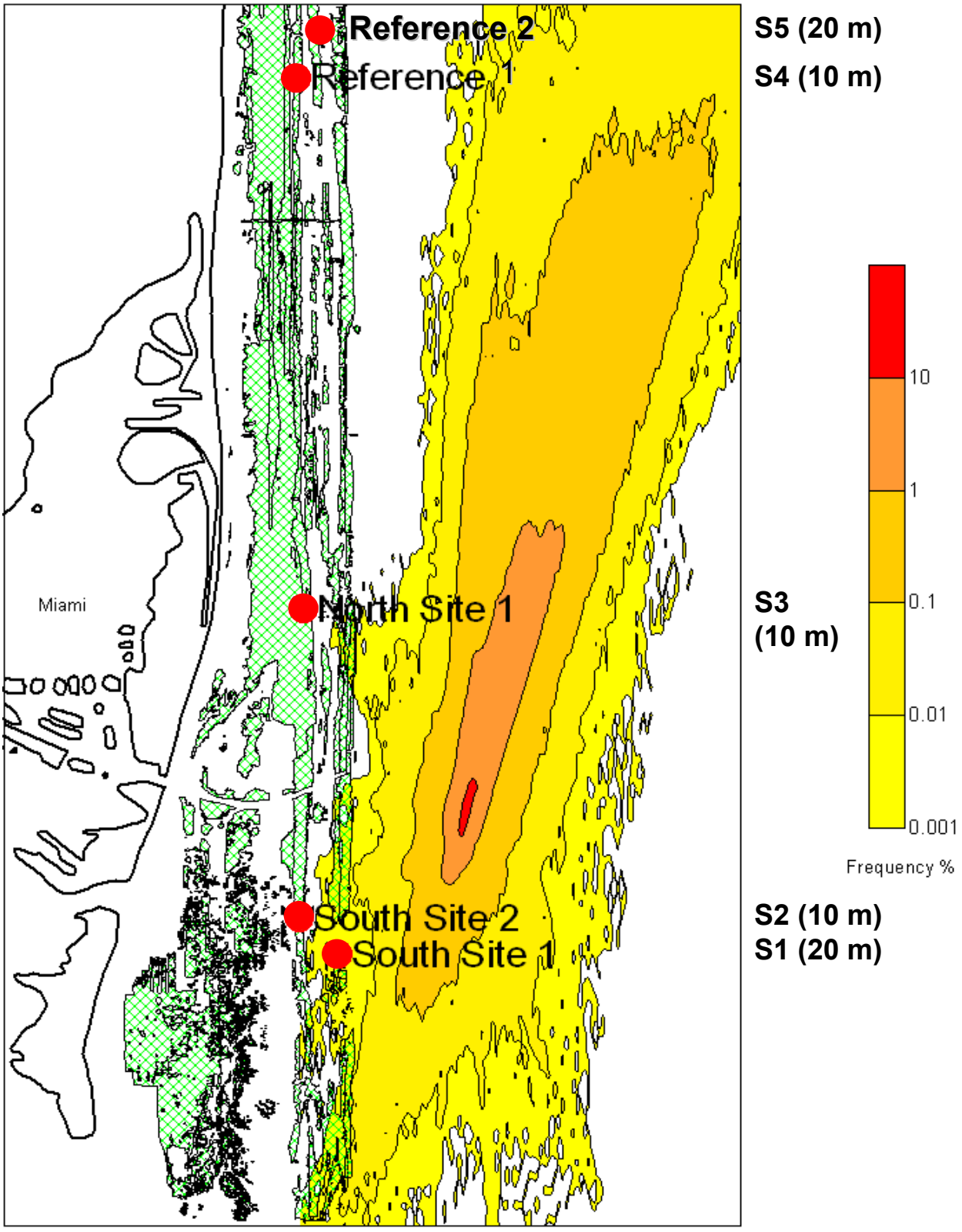


Figure 2. Image of barge dumping dredged material offshore.

Figure 3. Photograph of coral colony before dredge material dumping in June 2005 and after dredge material dumping in August 2005.

Montastrea cavernosa **coral colony from South Site 1, June 2005 (BEFORE dredging)**

Montastrea cavernosa **coral colony from South Site 1, August 2005 (AFTER dredging)**

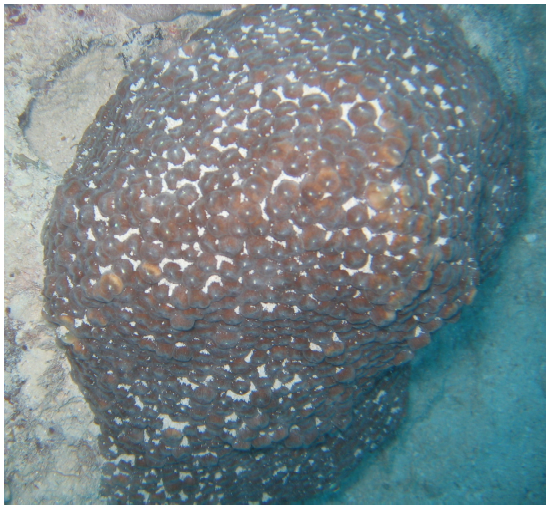


Figure 4. Primary Y axis: Graph of dumping activity measured in foot change of barge draft between full load upon departure and empty vessel upon return. Secondary Y axis: Graph of ocean temperatures (OTMP) measured with EPA Acoustic Doppler Current Profiler (ADCP) near bottom of South Site 1. Grey bars indicate collection dates. C1) February 22nd – 24th, 2005; C2) June 20th – 22nd, 2005; C3) August 16th – 18th, 2005; C4) October 10th – 13th, 2005; C5) June 19th – 22nd, 2006. Temperatures in parentheses are the average ocean temperatures during the respective collection dates. Dumping of dredge material commenced on July 11th, 2005.

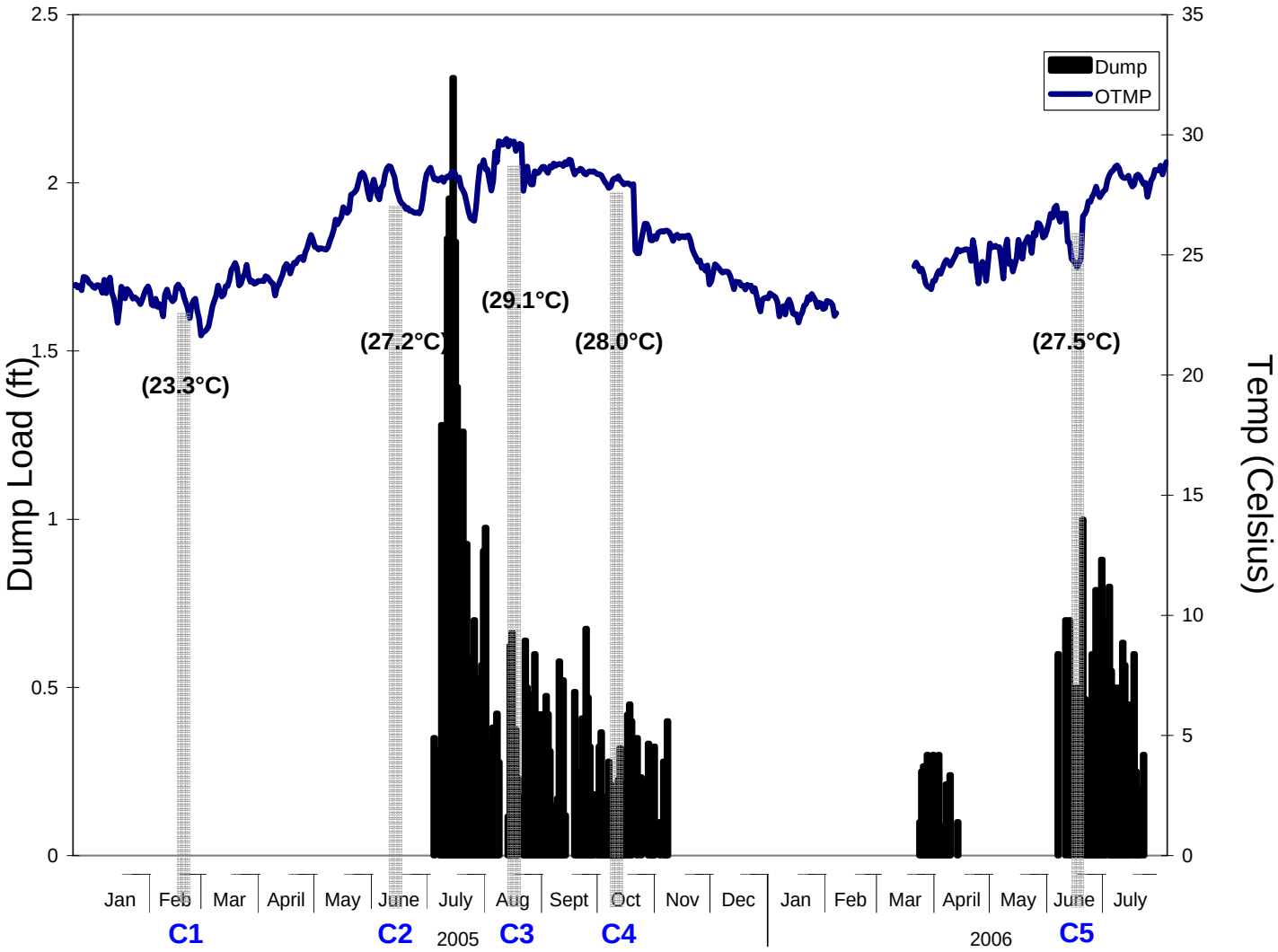


Figure 5. Representative colonies from each site and collection date for 2005.

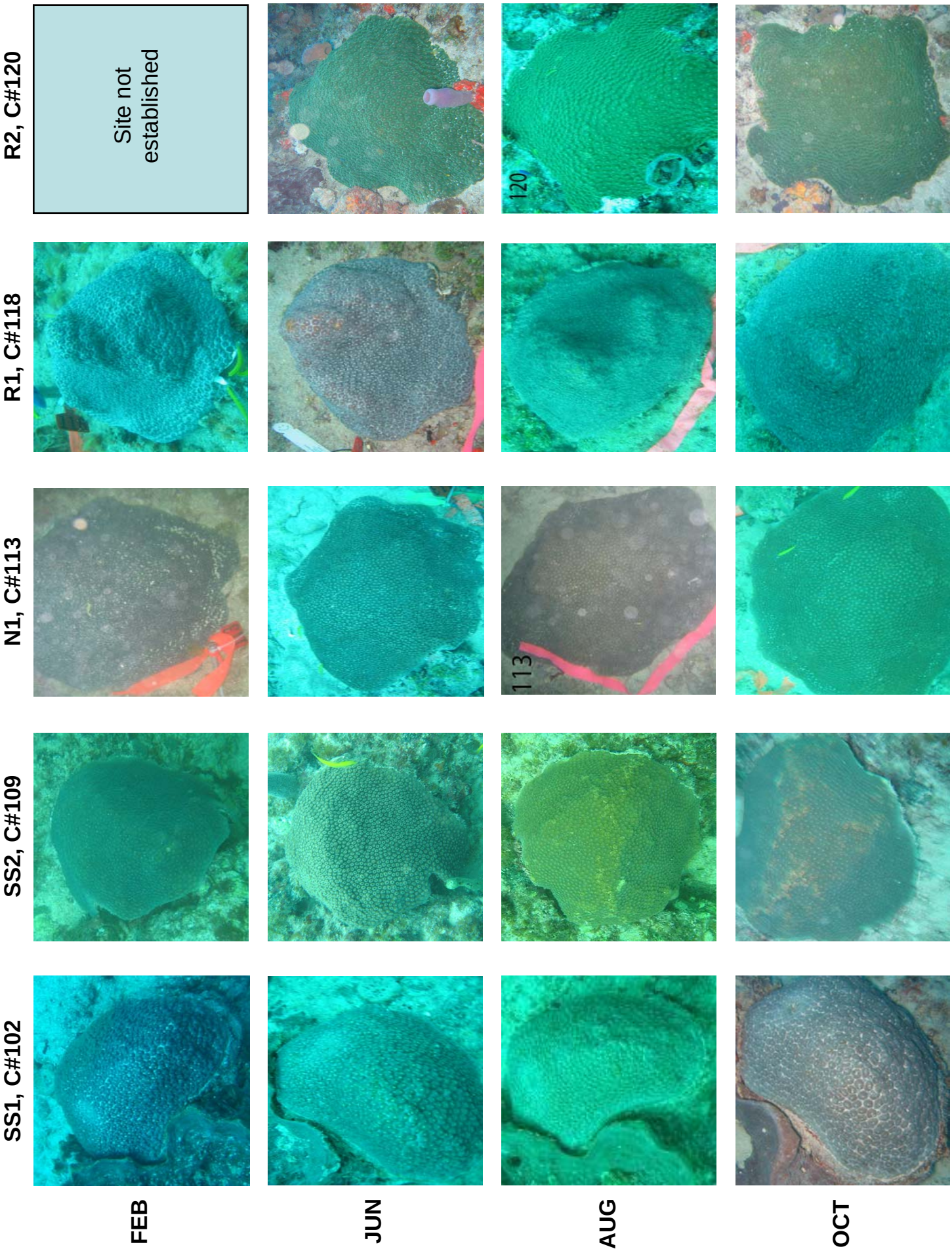


Table 2. Grouping of stress genes based on Gene Ontology and number of genes represented in each group.

Subgroup	**# of Gene in Group**
General Cellular Defense	11
Heat Shock Elements	11
Response to High Light / Illumination	4
Inflammation & Wounding	14
Response to Low Light / Shading	4
Oxidative Stress	19
Regulation of Apoptosis	6
Response to UV Radiation Exposure	4
Xenobiotic / Heavy Metal Exposure	15

Figure 6. The sum of expression level values for all genes by ***site***. Results indicate that colonies from South Sites 1 and 2 exhibited higher levels of gene expression compared to other sites. Colonies from Reference Site 2 reveal a lower level of expression than any other site.

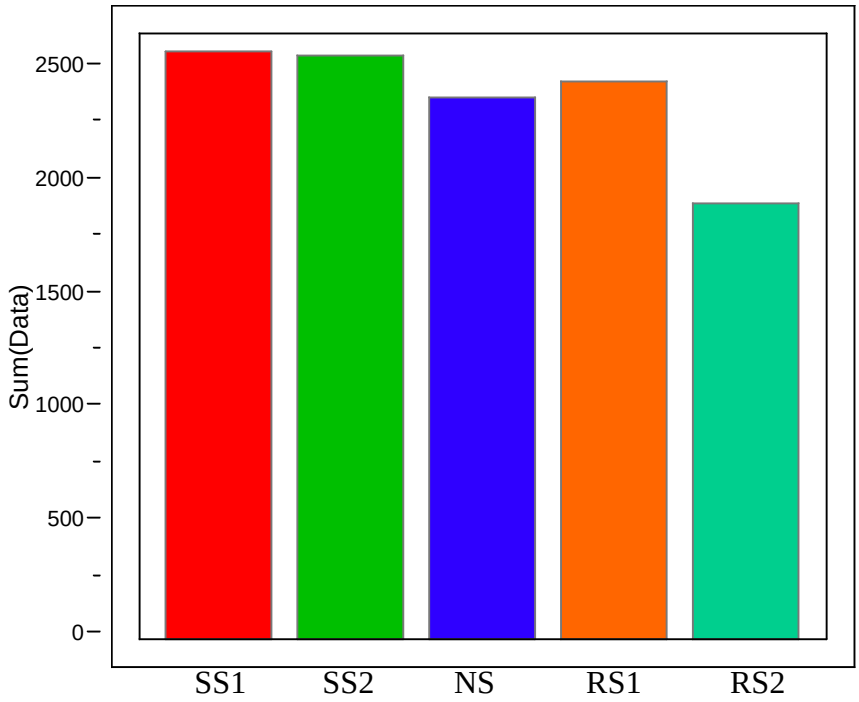


Figure 7. The sum of expression level values for all genes by ***date***. Results indicate that colonies from the February 2005 collection exhibit lower level of gene expression than any other date.

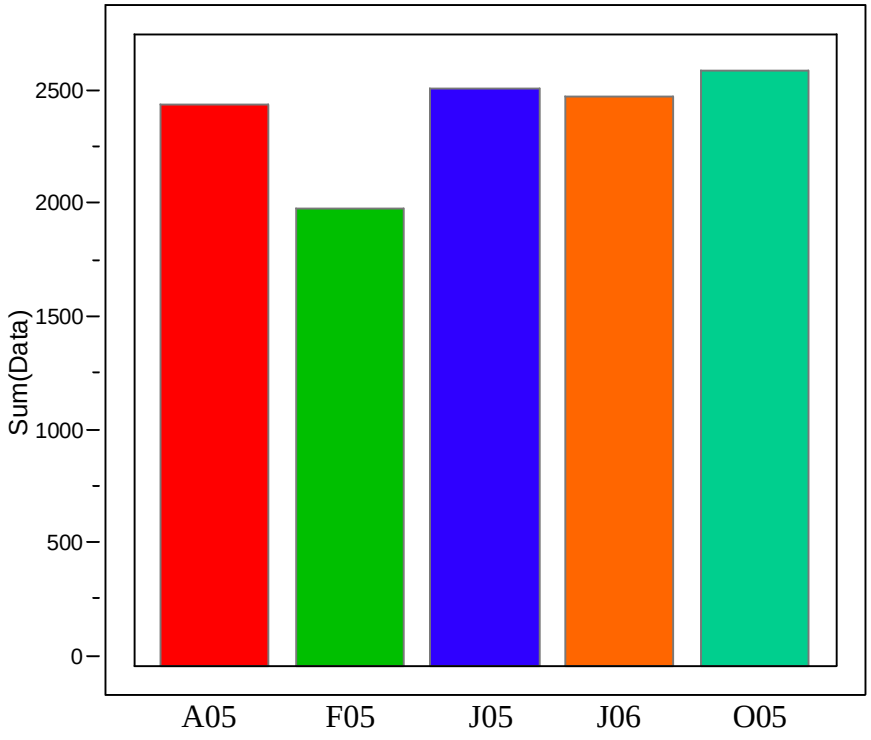


Figure 8. Relative gene expression of all genes by date and site. Solid color = stress genes. Hatched pattern = non-stress genes. Red = elevated expression; Green = decreased expression; Grey = non-significant expression (no change between sites or dates). **a)** Grouping of February and June 2005 based on similarity in expression patterns. **b)** Grouping of August and October 2005 based on similarity of expression patterns. **c)** June 2006 expression patterns.

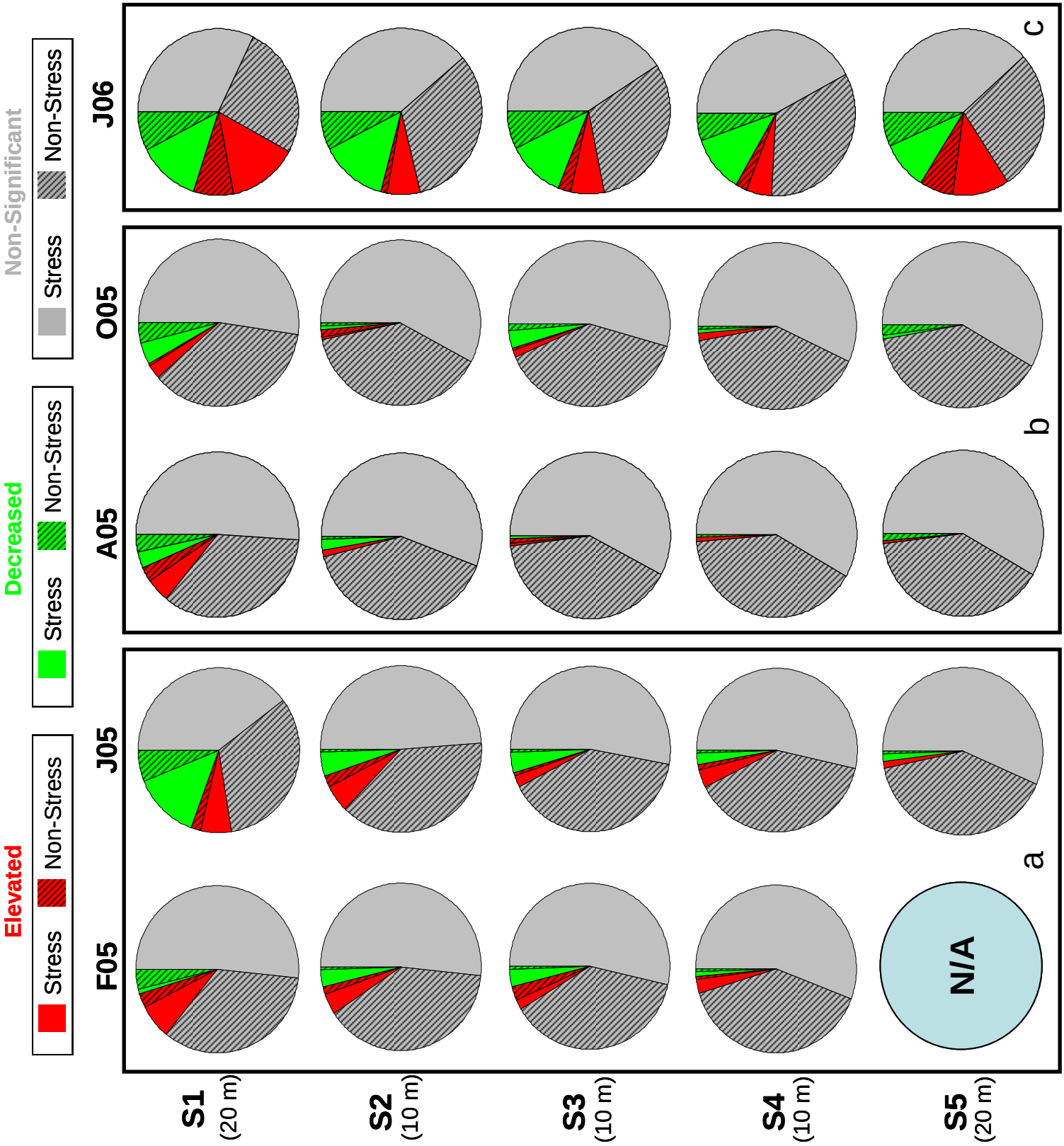


Figure 9. Relative gene expression. Solid color = stress genes. Hatched = non-stress genes. Red = elevated expression; Green = decreased expression; Grey = non-significant expression.
a) Grouping of Sites 2 – 5 based on similarity in expression patterns. **b)** Site 1 expression patterns.
c) Percent of *elevated stress genes* at each site.

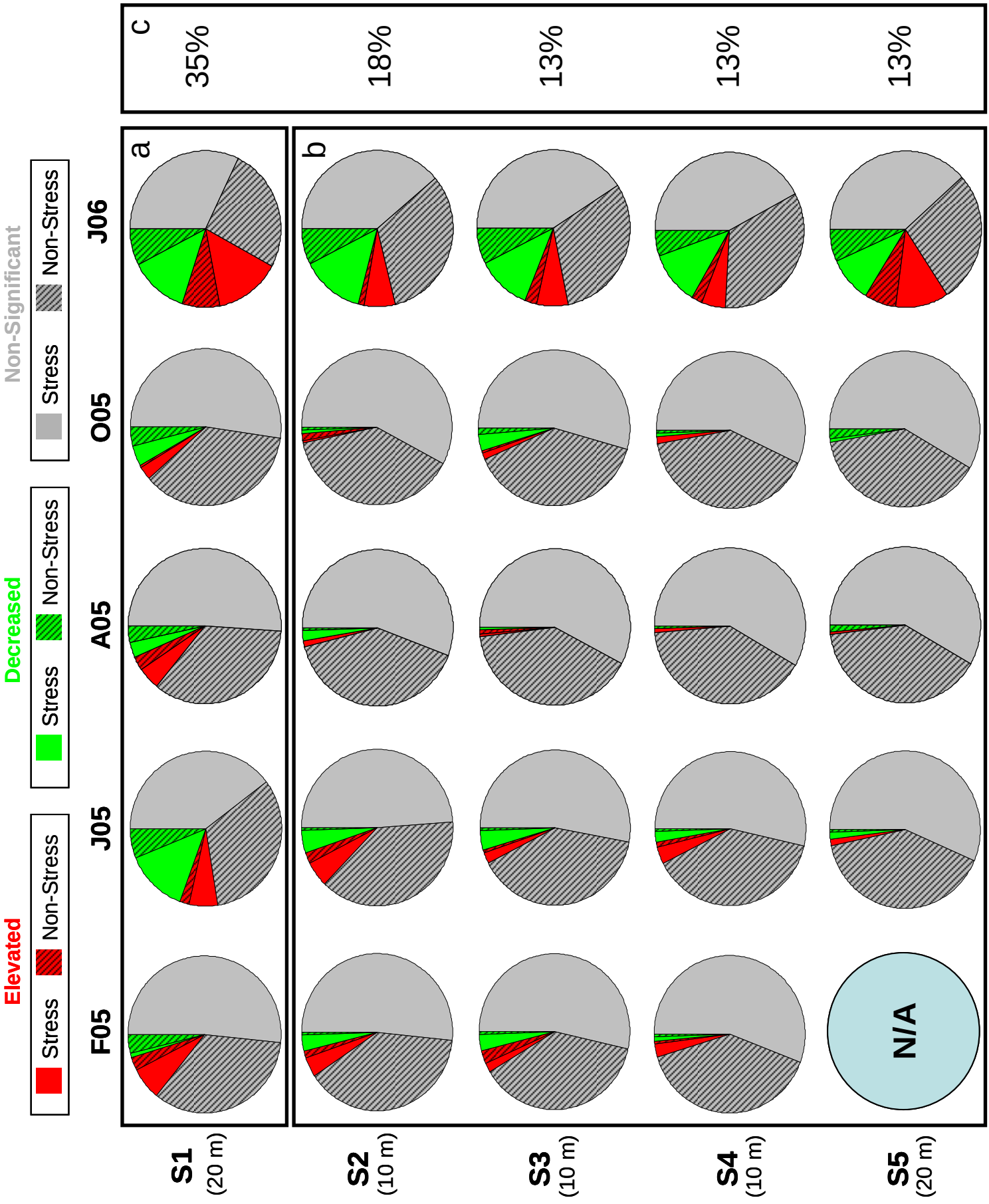


Figure 10. Relatively elevated genes in February 2005 by site. A) South Site 1 (20 m), B) South Site 2 (10 m), C) North Site (10 m), D) Reference Site 1 (10 m). *Reference Site 2 not established yet.*

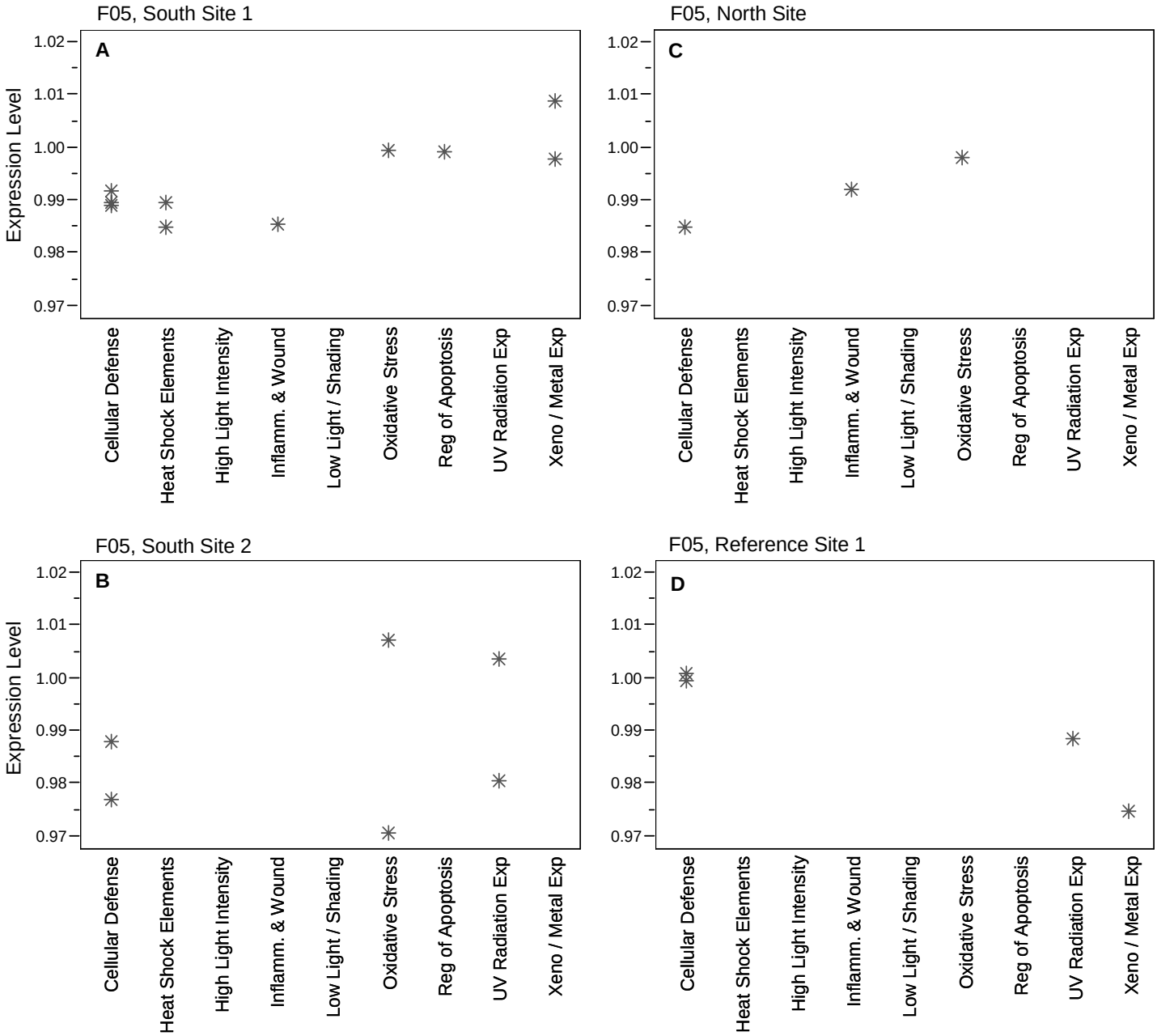


Figure 11. Relatively elevated genes in June 2005 by site. A) South Site 1 (20 m), B) South Site 2 (10 m), C) North Site (10 m), D) Reference Site 1 (10 m), E) Reference Site 2 (20 m).

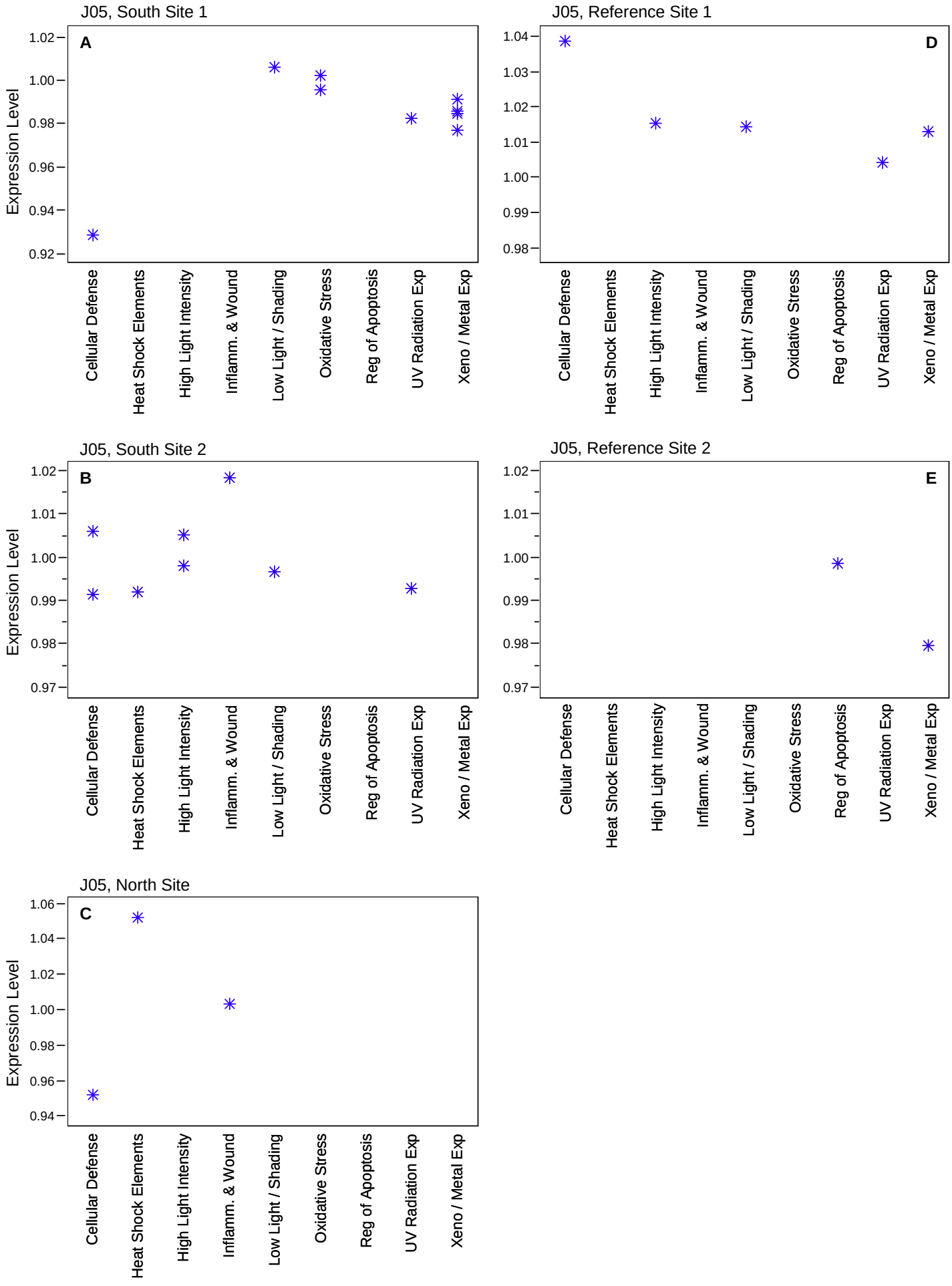


Figure 12. Relatively elevated genes in August 2005 by site. A) South Site 1 (20 m), B) South Site 2 (10 m), C) North Site (10 m), D) Reference Site 1 (10 m), E) Reference Site 2 (20 m).

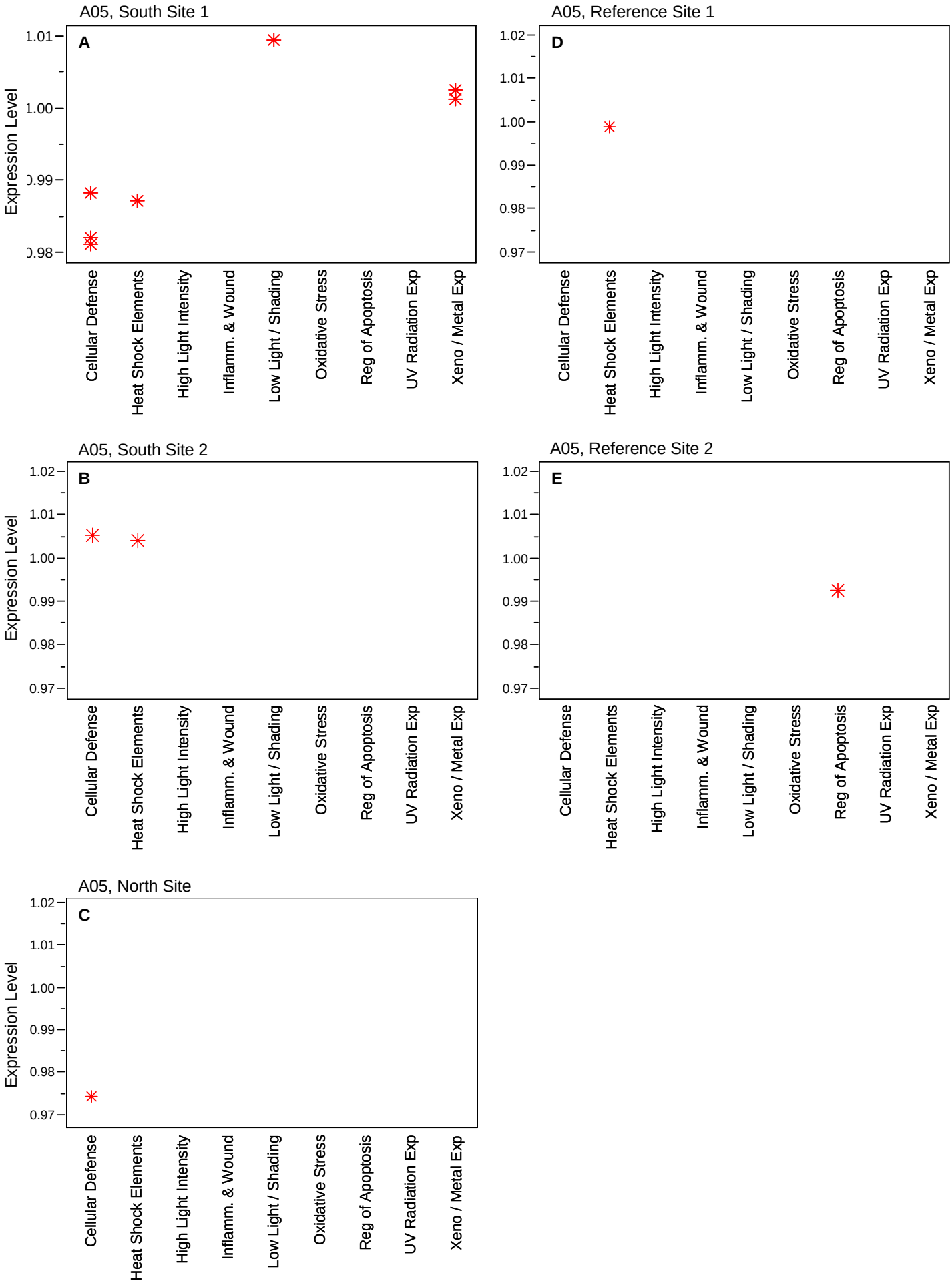


Figure 13. Relatively elevated genes in October 2005 by site. A) South Site 1 (20 m), B) South Site 2 (10 m), C) North Site (10 m), D) Reference Site 1 (10 m). *No significantly elevated genes at Reference Site 2 (20 m)*.

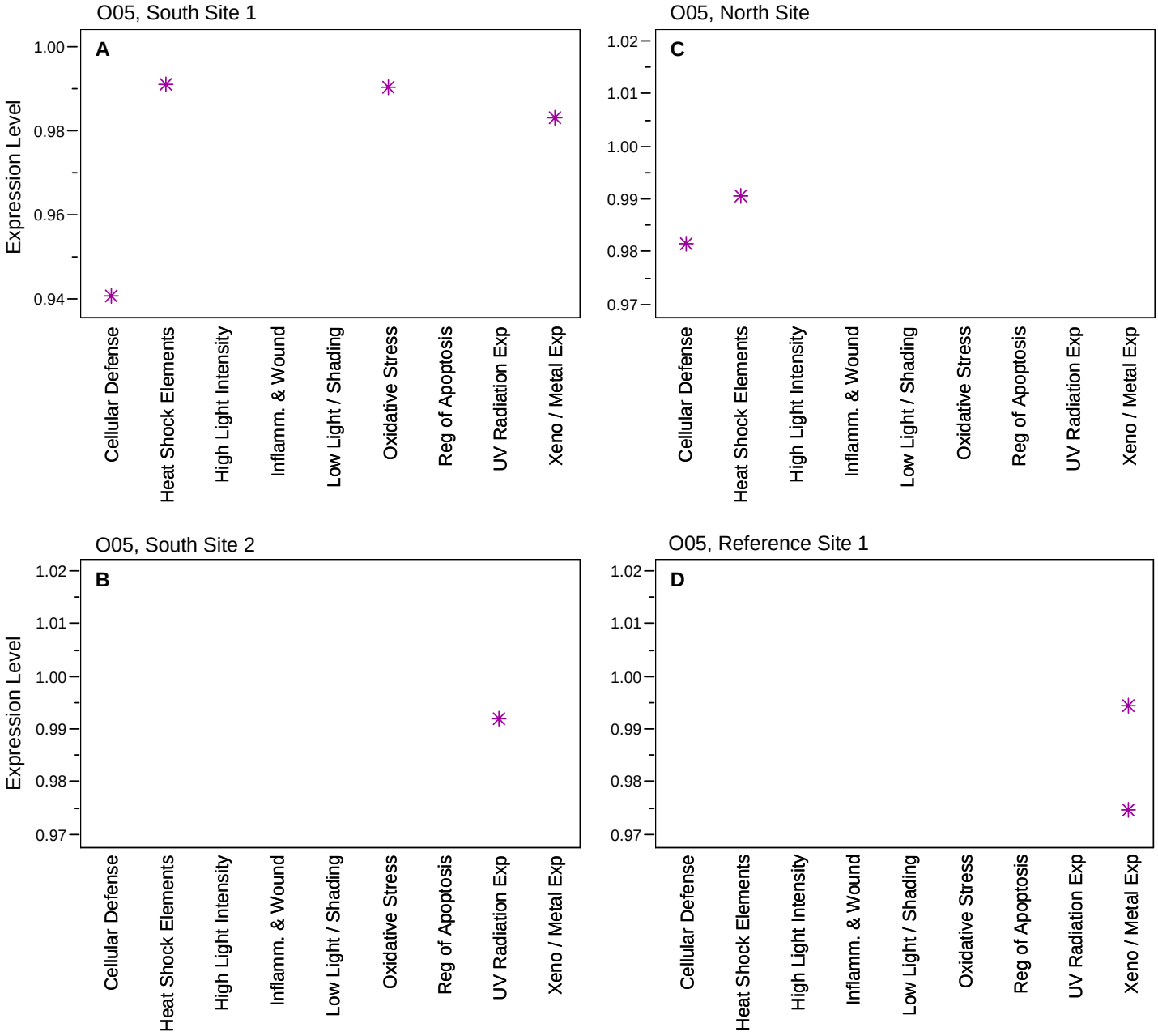
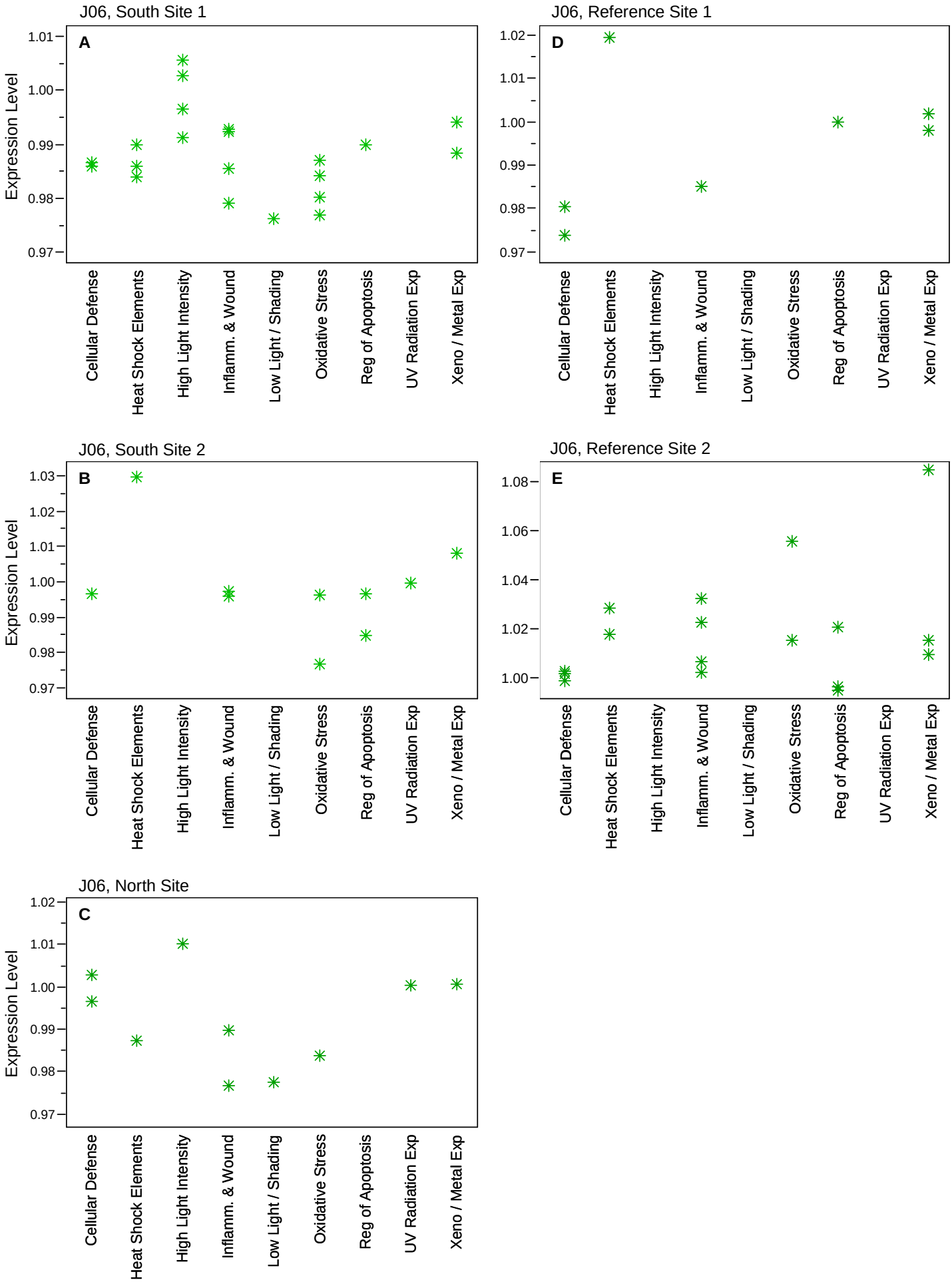


Figure 14. Relatively elevated genes in June 2006 by site. A) South Site 1 (20 m), B) South Site 2 (10 m), C) North Site (10 m), D) Reference Site 1 (10 m), E) Reference Site 2 (20 m).



Appendix A. Coral histopathological report by Bernarda Vargas-Angel.

EPA Port Of Miami Coral Study Histopathological Examination
Recorded by: Bernardo Vargas-Angel

Histopathological condition of tissues was ranked on a scale of 0 to 4, considering the type and severity of the tissue and cell alterations detected and their possible effect on the overall ability of corals to function normally. Specimens with clear structural integrity and no apparent injury are rated as healthy (0.0); those with slight to moderate parasite infections, algal/fungal/sponge infiltrates, slight tissue swelling, and/or lesions such as flattened epidermis, cellular debris, or minor tissue necrosis are described as mildly stressed (0.5–1.0). Those with extensive infections or infiltrates, lesions, and/or degenerative changes of reproductive products/gamete resorption, are considered as moderately stressed (1.5–2.0). Those specimens with loss of mucous secretory cells and large areas of tissue necrosis are considered to be markedly stressed (2.5–3.0). Finally, specimens exhibiting extensive necrosis, breakdown of normal cell architecture, suggestive irreversible changes, are rated 3.5–4.0.

Coral tissue and system codes: EP: Epidermis, GD: Gastrodrmis, CO: Coenosarc, CE: Calicoblastic epithelium, GC: Gland Cells, ZOOX: Zooxanthellae, CGB: Cnidoglanular band, OD: Oral Disk, SP: Spirocysts, MF: Mesenterial Filaments, MS: Mesenteries, MSC: Mucus secretory cells, BA Bacterial aggregates, GVC: Gastrovascular cavity, NP: Not present.

Site and Colony ID	Slide ID	Reproductive Status	Condition score	Comments
South Site 1 Colony 101	A001	Stage II oocytes	1.5	Focal to multi-focal disruption of EP (collection) Normal tissue integrity, healthy-looking EP Normal appearance and number of MSC in EP, CO, OD, and MS. CE wispy and cuboidal-looking Diffuse presence of algal infiltrates. Degenerative Stage II oocytes Focal to multi-focal granular, necrotic spots in CGB, and MF Focal increase in numbers of eosinophilic GC in MS
South Site 1 Colony 102	A002	NP	1.0	Focal to multi-focal tissue disruption (collection artifact) Diffuse presence of mucin in GVC (collection artifact) Normal tissue integrity, healthy-looking EP, CO, OD

				Normal appearance and number of MSC in EP, CO, OD, and MS. CE wispy and cuboidal-looking Focal to multi-focal presence of algal and sponge infiltrates. Focal increase in numbers of eosinophilic GC in MS Focal to multi-focal granular, necrotic spots in CGB, and MF
South Site 1 Colony 103	A003	NP	1.0	Overall normal tissue integrity and healthy-looking. EP clear and thinned-out in localized areas. Normal appearance and number of MSC in EP, CO, OD, and MS. CE granular and wispy. Focal to multi-focal presence of algal infiltrates. Focal to multi-focal increased numbers of eosinophilic GC in CGB and MF. Focal to multi-focal granular, necrotic spots in CGB and MF.
South Site 1 Colony 104	A004	Stage II oocytes	1.5	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact) EP clear and thinned-out in localized areas. Normal appearance of MSC in EP, CO, OD, and MS. Low numbers of MSC in EP and CO. Focal to multi-focal granular and wispy appearance of CE. Focal to multi-focal granular necrotic spots in CGB, and MF Focal degenerative Stage II oocytes; uneven vitellogenesis/granularity.
South Site 2 Colony 105	A005	Stage II-III oocytes	1.5	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact) Normal appearance of MSC in EP, CO, OD, and MS. Focal to multi-focal algal infiltrates. Focal to multi-focal granular and wispy appearance of CE. Focal to multi-focal granular necrotic spots in CGB, and MF Focal degenerative Stage II-III oocytes; uneven vitellogenesis/granularity.

South Site 1 Colony 106	Missing	NA	NA	NA
South Site 2 Colony 107	A007	NP	0.5	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact) Normal appearance of MSC in EP, CO, OD, and MS. Focal to multi-focal granular and wispy appearance of CE. Focal granularity in middle polyp region. Focal granularity in middle polyp region Focal increased number of eosinophilic GC in middle polyp region.
South Site 2 Colony 108	A008	NP	0.5	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact) Normal appearance of MSC in EP, CO, OD, and MS. Low numbers of MSC in EP and CO. Multi-focal to diffuse algal infiltrates. Focal increased number of SP in CGB.
South Site 2 Colony 109	A009	NP	0.5	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact) Focal to multi-focal disruption of EP (collection) Normal appearance of MSC in EP, CO, OD, and MS. Multi-focal algal infiltrates. Focal increased number of eosinophilic GC in middle polyp region.
North Site 1 Colony 110	A010	NP	1.0	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact) Focal to multi-focal disruption of EP (collection) Normal appearance of MSC in EP, CO, OD, and MS. Focal increased number of eosinophilic GC in MF Focal increased number of SP in CGB and MF Focal to multi-focal granular necrotic spots in CGB and MF
North Site 1 Colony 111	A011	Stage II-III oocytes	2.0	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact)

				Focal to multi-focal disruption of EP (collection) Normal appearance of MSC in EP, CO, OD, and MS. Focal to multi-focal presence of algal infiltrates. Focal necrotic damage to tentacles. Focal to multi-focal degenerative change to stage II-III oocytes Focal increased number of SP in CGB and MF Focal to multi-focal granular necrotic spots in CGB and MF Focal pyknotic spots in CGB and MF.
North Site 1 Colony 112	A012	Stage III oocytes	1.5	Overall normal tissue integrity and healthy-looking. Diffuse presence of mucin in GVC (collection artifact) Focal to multi-focal tissue disruption (collection). Normal appearance of MSC in EP, CO, OD, and MS. Focal to multi-focal presence of algal infiltrates. Focal presence of metazoan infilatrates. Focal to multi-focal degenerative change to stage II-III oocytes Focal to multi-focal increase of eosinophilic GC in MF
North Site 1 Colony 113	A013	Stage III oocytes	1.0	Overall normal tissue integrity and healthy-looking. Normal appearance of MSC in EP, CO, OD, and MS. Focal to multi-focal presence of algal infiltrates. Focal to multi-focal granular necrotic spots in CGB and MF x 2 Multi-focal granularity in lower polyp region x 1 polyp. Multi-focal granularity in middle polyp region x 2 polyp
North Site 1 Colony 114	A014	Stage III oocytes	3.0	Diffuse presence of mucin in GVC (collection artifact) Focal to multi-focal tissue disruption (collection). Overall normal tissue integrity and healthy-looking. Normal appearance of MSC in EP, CO, OD, and MS. Focal necrotic damage to tentacles x 1 polyp Focal to multi-focal granular, necrotic spots in CGB and MF x 2-3 x 3 polyps Multi-focal granularity in middle polyp region x 2 polyp